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ORIGINAL ARTICLE

Design, synthesis and biological evaluation of novel quinazoline derivatives as potential NF-κb inhibitors

Jing Pan [a], Long Ma [a], Yu-Xia Tang [a], Ying Tian [a], Yi-Hang Lin [a], Long-Jiang Zhang [a,*], Feng Gao [b,*], Guang-Ming Lu [a,*]

[a] *Department of Medical Imaging, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China*
[b] *Haina Precision Machining Research Center, Yangtze Delta Region Research Institute of Tsinghua University, Zhejiang, China*

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Abstract A series of novel 4-aminoquinazoline derivatives were designed, synthesized and biological properties on nuclear factor-kappaB (NF-κb) pathway inhibitory and potential in vitro anti-proliferation against breast cancer lines were also evaluated. Among them, ***LU1501*** exhibited potent inhibition with IC_{50} values in SK-BR-3 (10.16 ± 0.86 μM) and HCC1806 (10.66 ± 1.01 μM) cell lines. In vivo studies in breast cancer tumor model proved the correlation between anticancer activity of ***LU1501*** and proliferation inhibition through the NF-κb signal pathway. The molecular docking studies also portrayed the potential binding mechanism between ***LU1501*** and the key proteins of p65 and IkBα in NF-κb pathway. Accordingly, compound ***LU1501*** could serve as a potent agent against breast cancer for further investigation.

1. Introduction

Tyrosine Kinase (TK) is a tyrosine-specific protein kinase and its receptor like epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor-1(IGFR-1) and hepatic growth factor receptor (HGFR) (Yin et al., 2019; Sun et al., 2020) could bind to a ligand and phosphorylates tyrosine residues. The EGFR TK family includes four receptor tyrosine kinases, namely, EGFR (HER1), ERBB-2 (HER2), ERBB-3 (HER3), and ERBB-4 (HER4). EGFR, a transmembrane protein, is the first tumor-associated cell surface receptor (Sassen et al., 2008; Jiang et al., 2021). Tyrosine kinase plays an important role in the occurrence and development of cancer and also represents an important target for cancer treatment (Fang et al., 2020; Cheng et al., 2021; Jiang et al., 2019). Dozens of drugs targeting TK have been designed and approved for clinical treatment of cancer. For example, erlotinib, which could selectively target EGFR, has been applied to treat non-small-cell lung carcinoma (non-small cell lung cancer) in clinics; and lapatinib, the HER2 targeted drug, is highly effective against breast cancer (Roskoski, 2019; Wu et al., 2021; Ding et al., 2018).

* Corresponding authors.
E-mail addresses: kevinzhlj@163.com (L.-J. Zhang), rggaofeng@hotmail.com (F. Gao), cjr.luguangming@vip.163.com (G.-M. Lu).
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Table 1 Yield and purity of synthesized compounds.

Compound	Yield (%)	Purity (%)
LU1501	41	98.0
5b	26	99.3
5c	54	98.1
5d	80	98.0
5e	22	95.2
9f	33	96.6
9g	26	99.9
9h	36	91.9
9i	57	99.2
9j	7	92.1

Small molecular tyrosine-kinase inhibitors (TKI) can be divided into quinazoline, quinoline, pyrimidine, pyrimidine, and indole (Han et al., 2021; Sayed et al., 2018; Faisal and Saeed, 2021). Quinazolines are a class of compounds with a wide range of biological activities, such as anti-cancer, anti-bacterial, anti-inflammatory, anti-malaria, anti-hypertension and other effects (Abuelizz et al., 2017; Yang et al., 2014; Krapf and Wiese, 2016). Quinazoline derivatives could inhibit EGFR or EGF receptor tyrosine kinase in Pro-epidermal growth factor cells, resulting in anti-cancer activity, and therefore they could be used to fight prostate cancer, lung cancer, gastric cancer and bile cancer. At present, over 40 quinazoline inhibitors, such as gefitinib, erlotinib, afatinib, lapatinib, icotinib, vandetanib and so on, have been applied in clinics (Alagarsamy et al., 2018; Wdowiak et al., 2021; Ashmawy et al., 2020).

Quinazoline inhibitors have been shown to possess several attractive pharmacological activities. Para-aryl modification of the 4-aminoquinazoline ring account for the vast majority of anticancer

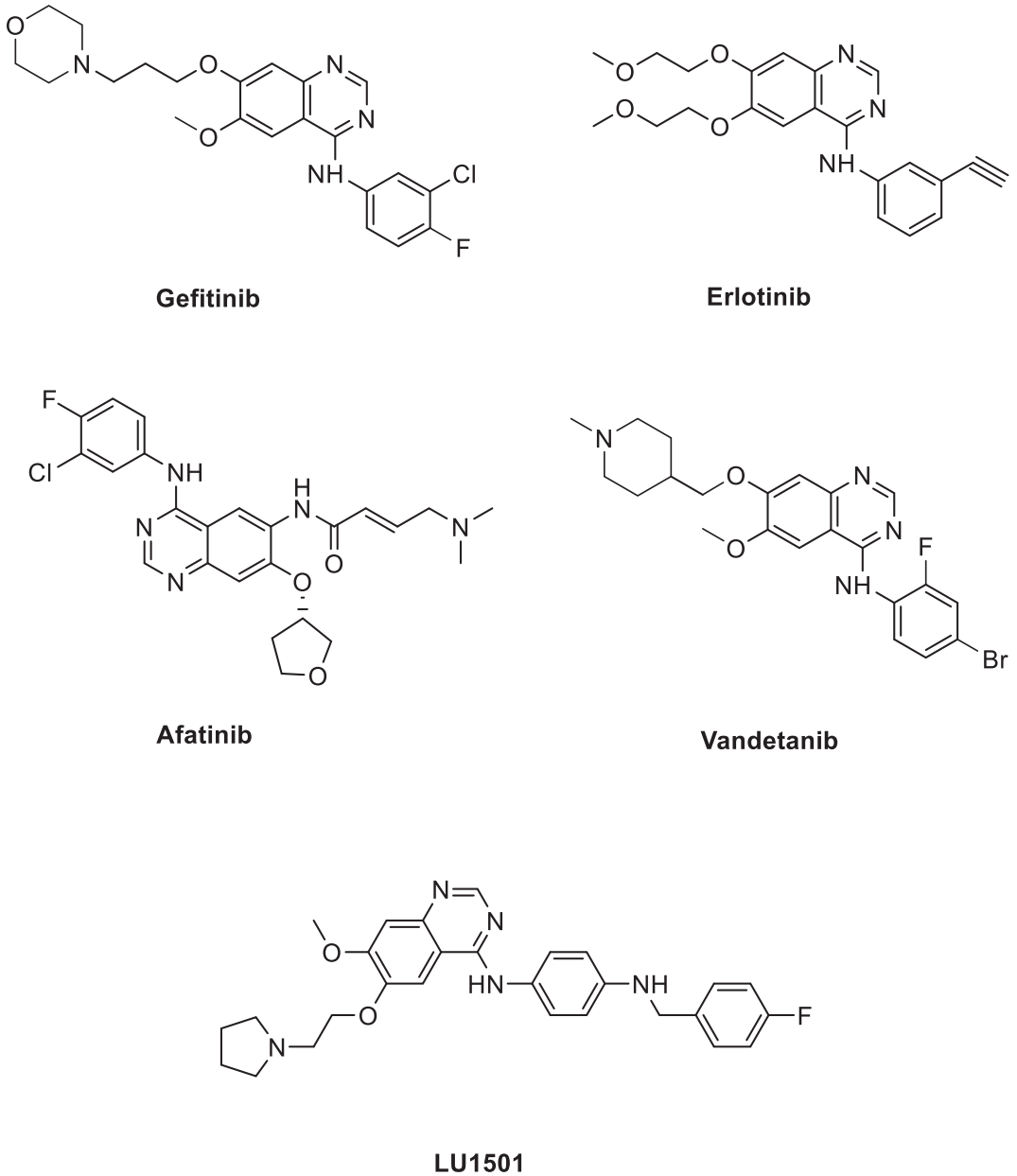
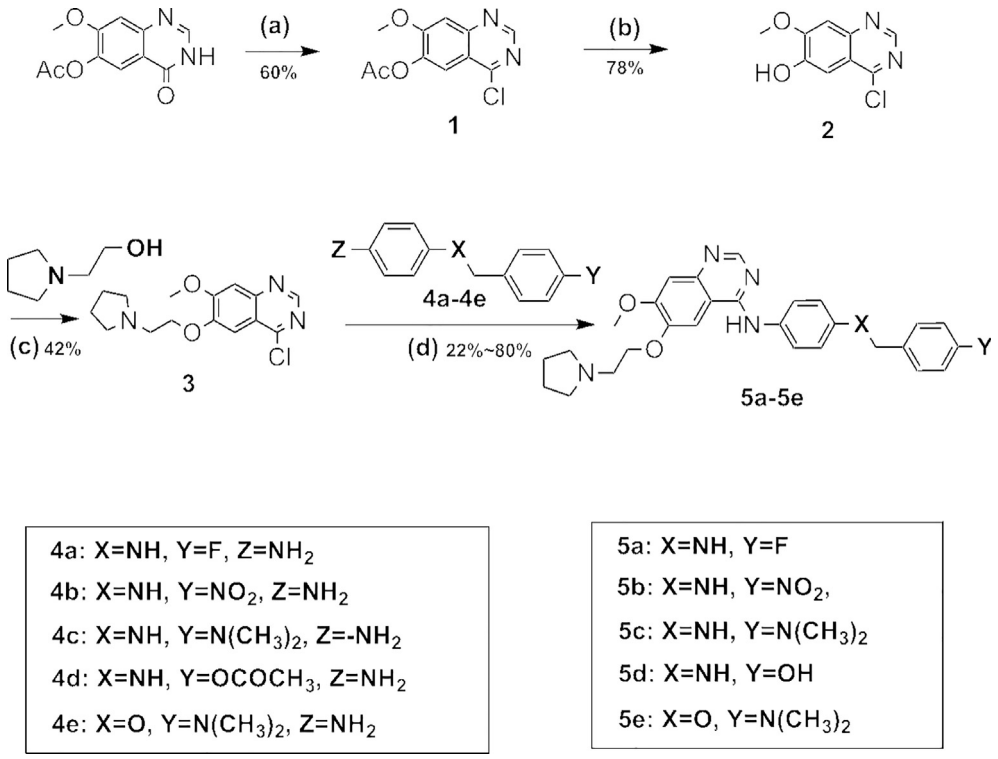
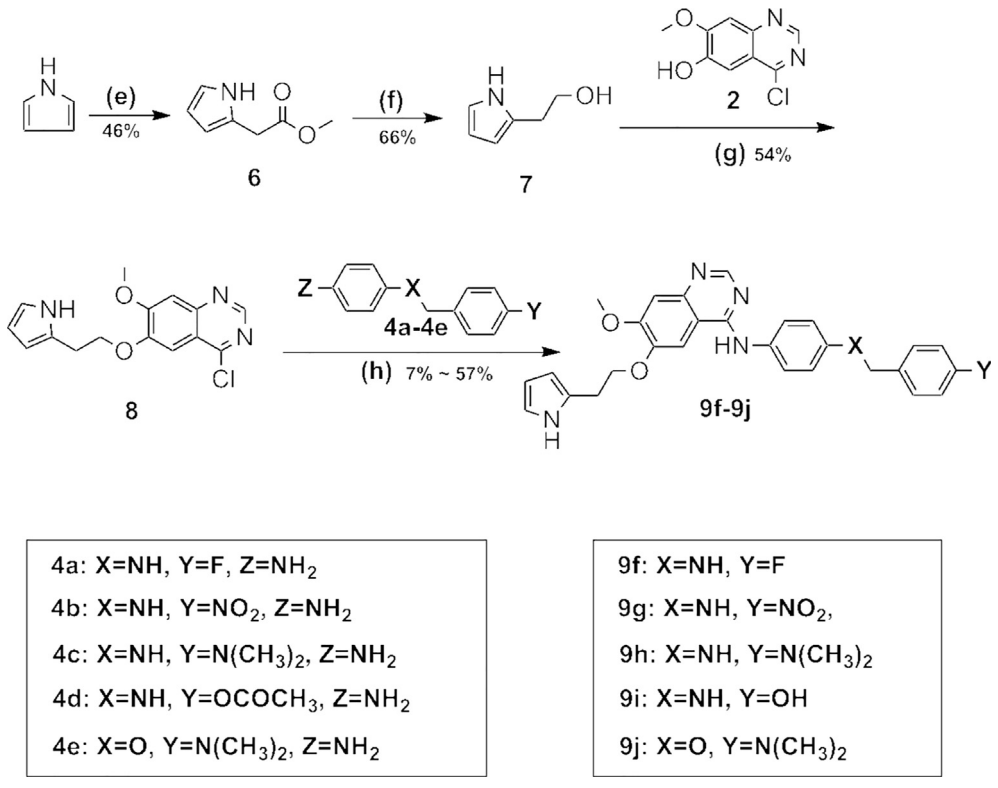


Fig. 1 Chemical structures of representative quinazoline inhibitors and the target compound ***LU1501***.



Scheme 1 Synthetic procedure for compounds ***5a–5e.***



Scheme 2 Synthetic procedure for compounds ***9f–9j.***

Table 2 IC_{50} values of ***LU1501*** against five cells for 48 h in vitro.

Compound	IC_{50} (μM)				
	MDA-MB-468	MCF-7	SK-BR-3	HCC1806	HEK 293 T
LU1501	19.45 ± 1.33	16.13 ± 3.47	10.16 ± 0.86	10.66 ± 1.01	>100
5b	26.91 ± 1.74	4.06 ± 1.08	10.41 ± 1.13	14.35 ± 1.31	>100
5c	4.68 ± 0.43	33.33 ± 3.15	3.95 ± 0.46	25.60 ± 2.18	>100
5d	34.32 ± 1.89	> 100	51.29 ± 3.87	47.32 ± 1.54	>100
5e	52.33 ± 7.56	49.66 ± 6.87	60.31 ± 9.31	> 100	>100
9f	5.22 ± 1.68	32.54 ± 3.81	12.30 ± 1.02	21.78 ± 3.11	>100
9g	17.48 ± 1.29	41.03 ± 6.50	31.27 ± 2.36	37.58 ± 1.22	>100
9h	21.02 ± 3.34	89.22 ± 6.17	25.26 ± 4.49	>100	>100
9i	24.49 ± 2.89	40.68 ± 1.81	27.26 ± 4.16	34.57 ± 1.29	>100
9j	10.26 ± 1.08	54.69 ± 3.79	23.73 ± 4.03	33.25 ± 1.18	>100
Elotinib	16.1 ± 1.46	23.81 ± 1.25	51.65 ± 0.38	>100	>100

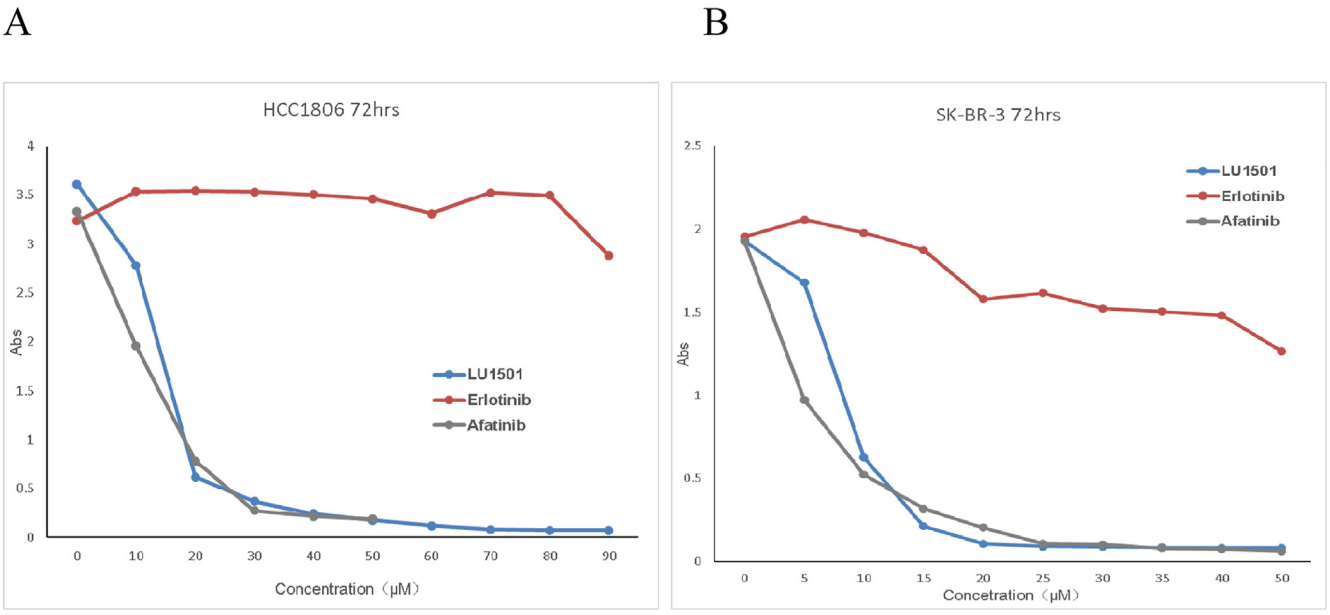


Fig. 2 Compound ***LU1501***, Elotinib and Afatinib inhibit proliferation in breast cancer cell lines SK-BR-3 and HCC1806 after treated for 72hrs. (A) HCC1806 cell lines (B) SK-BR-3 cell lines.

activities. Based on the above analysis and the structures of newly developed clinical drugs (Fig. 1), we thought modification of *para*-aryl of the 4-aminoquinazoline ring was another clue for designing new derivatives.

Here, we reported a series of novel quinazoline derivatives, which can effectively inhibit the growth and proliferation of breast cancer cells by suppressing the activation of NF-κb signaling pathway, especially HER-2 and EGFR-positive cell lines such as SK-BR-3.

2. Results

2.1. Chemistry

The preparation routes of the target compounds were shown in Scheme 1 and Scheme 2. The preparation yield and purity of the target compounds were shown in Table 1. Compound ***1*** was obtained successfully by adding 7-methoxy-4-oxo-3,4-dihydroquinazolin-6-yl acetate, sulfurooyl dichloride into N, N-dimethylformamide dropwise in an atmosphere of nitrogen, Then compound ***1*** reacted with NH_3 in methanol, leading to compound ***2***. Later, conjugating compound ***2*** with 2-(pyrrolidin-1-yl)ethan-1-ol in tetrahydrofuran under DTAD and PPh_3 gave compound ***3***. Finally, target compounds ***5a–5e*** were obtained by the reaction of compounds ***3*** and ***4a–4e*** in the presence of 4-methylbenzene-1-sulfonic acid, and propan-2-ol (Liang et al., 2021; Gao et al., 2019).

In order to get the target compounds ***9f–9j***, compound ***6*** was firstly synthesized from 1H-pyrrole and methyl 2-bromoacetate. Afterwards, compound ***6*** was reduced by $LiAlH_4$ to afford compound ***7***. Compound ***8*** was achieved via conjugation of compound ***2*** and ***7***, and finally compounds ***8*** reacted with materials ***4a–4e*** **under** trifluoroacetic acid, yielding the target compounds ***9f–9j***.

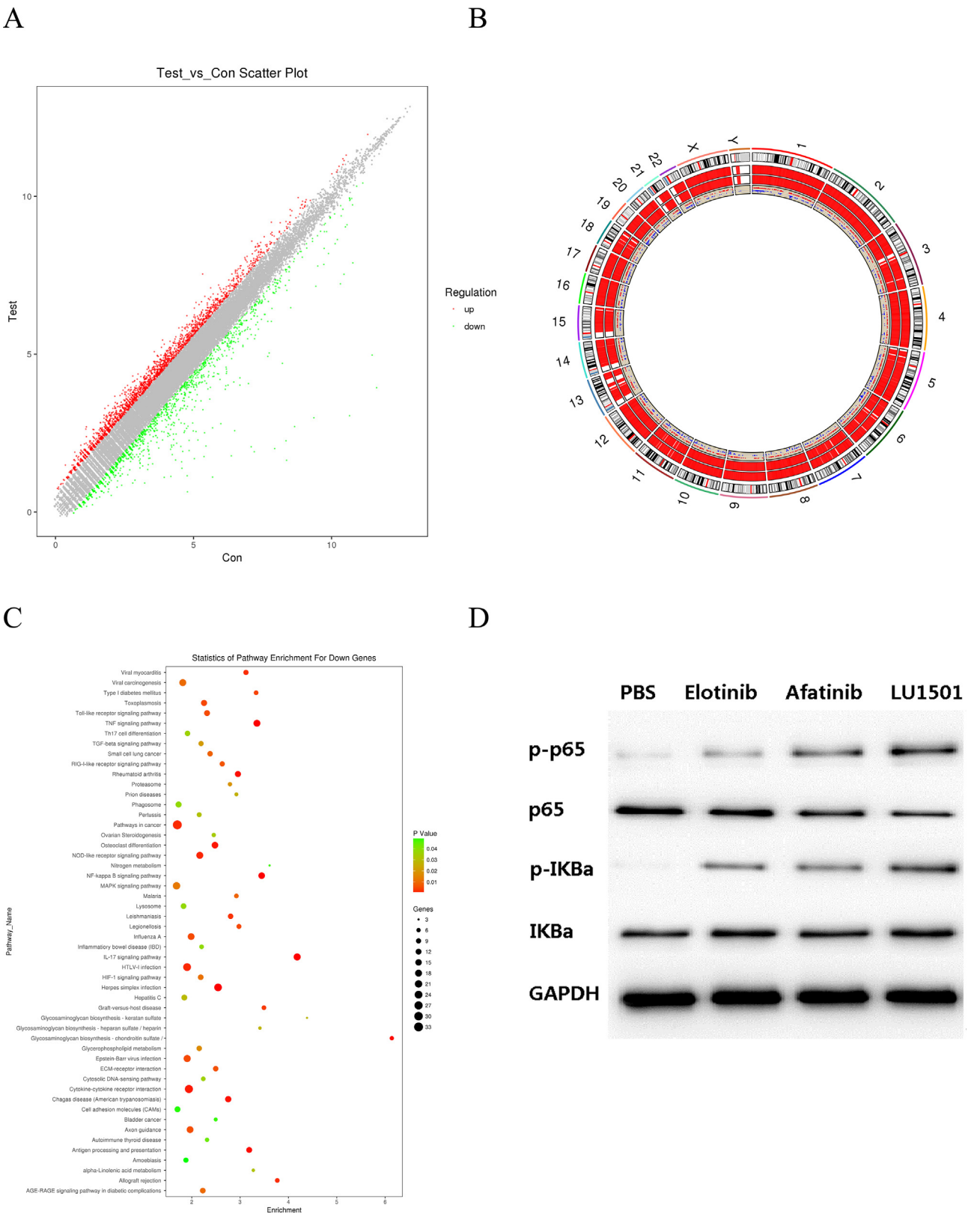


Fig. 3 ***LU1501*** inhibits EGFR/NF-κb signal pathway activity in SK-BR-3 cell line. (A) Scatter plot of differential genes showed genes up-regulated and down-regulated. (B) Differential genes were distributed specific positions on chromosomes. (C) NF-κb signaling pathway was enriched by the KEGG analysis. (D) The expression levels of EGFR/NF-κb pathway related proteins were evaluated using Western blot analysis. (E) Corresponding grayscale values analysis of EGFR/NF-κb p65 and phospho-p65. (F) Corresponding grayscale values analysis of EGFR/NF-κb IkBα and phospho-IkBα.

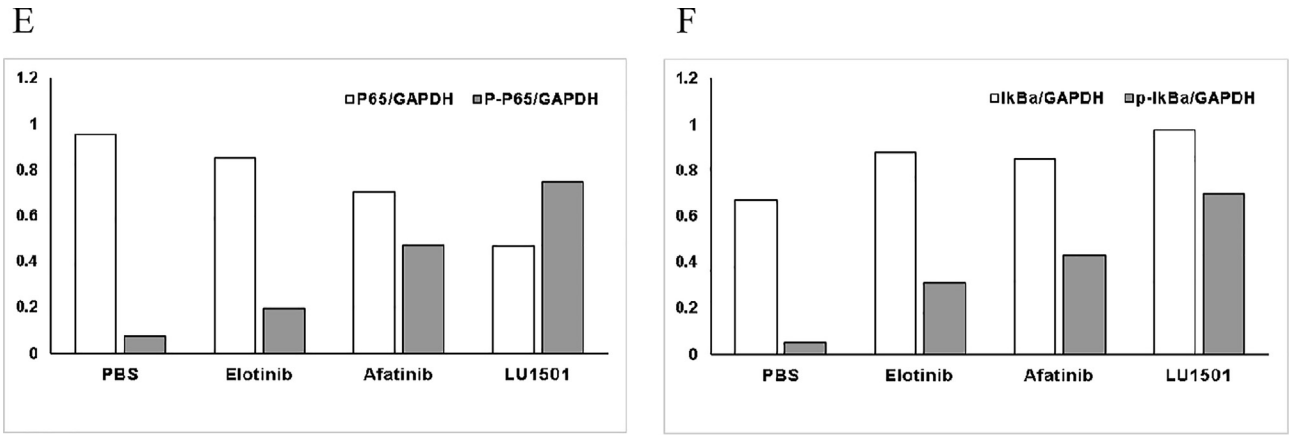


Fig. 3 (*continued*)

Reagents and conditions: (a) $SOCl_2$,DMF, 3h; (b) NH_3/MeOH, 10 °C; (c) PPh_3, DTAD, THF; (d) n-BuOH, TFA, 75 °C; (e) EtMgBr, THF,methyl 2-bromoacetate, HCl, 0 °C, 1 h; (f) THF, $LiAlH_4$, 0 °C; (g) PPh_3, DTAD, THF, r.t, overnight; (h) n-BuOH, TFA, 75 °C,1.5 h.

2.2. Biological evaluation

2.2.1. In vitro cytotoxic activity of novel quinazoline derivatives against MDA-MB-468, SK-BR-3, HCC1806, MCF-7 breast cancer cell lines and HEK 293T cells

The inhibitory activity of target compounds (IC_{50} ± SD μM) towards breast cancer cells was listed in Table 2. Erlotinib, a first-generation EGFR inhibitor, was used as a positive control. As shown in Table 2, the IC_{50} of compound ***5a*** (***LU1501***), ***5c***, ***9f***, ***9g***, ***9j*** was lower than 20 μM on MDA-MB-468. ***LU1501*** and ***5b*** showed strong inhibition on MCF-7 with $IC_{50} < 20$ μM. Additionally, ***LU1501***, ***5b***, ***5c***, ***9f*** displayed high cytotoxic activity on SK-BR-3 ($IC_{50} < 20$ μM) and ***LU1501*** together with ***5b*** could obviously suppress the proliferation of HCC1806 cells with $IC_{50} < 20$ μM. Generally, most of the tested target compounds seemed to be more effective against SK-BR-3 than against other cell lines. ***LU1501***, the most active compound among the tested derivatives, exhibited significantly lower IC_{50} values than the positive control on the MCF-7, SK-BR-3, and HCC1806 cells. Meanwile, compounds on HEK 293T cells displayed minimal toxicity at the dosage of 100 μM for 48hrs as shown in Table 2.

The cell viability and inhibition of cell proliferation, as well as dose–response curves of ***LU1501***, Elotinib and Afatinib on SK-BR-3 and HCC1806 breast cancer cell lines were illustrated in Fig. 2. The results showed that cell viability decreased nearly 100% with significant inhibition on HCC1806 and SK-BR-3 proliferation with ***LU1501*** treatment for 72 h, which was similar with the inhibition of Afatinib. Moreover, the inhibitory activity of ***LU1501*** **on** HCC1806 and SK-BR-3 cells was higher than that of Elotinib. All these indicated that ***LU1501*** possessed a potential anti-tumor effect on breast cancer, especially on HCC1806 and SK-BR-3 cell line.

A B.

2.2.2. *LU1501* inhibits EGFR/NF-κB signal pathway activity in SK-BR-3

Because ***LU1501*** demonstrated the highest anticancer activity among all the tested derivatives, we investigated the regulatory mechanism of ***LU1501*** in breast cancer cells by Agilent microarray analysis. From the IC_{50} value and cell viability curve results listed above, SK-BR-3 cell line was quite sensitive to ***LU1501***, therefore SK-BR-3 was used to explore the differentially expressed genes (DEGs) responding to ***LU1501*** treatment. As showed in Fig. 3**A–C, 534** genes were up-regulated and 1872 genes were down-regulated. NF-κb signaling pathway was enriched by the KEGG analysis. The protein expression was further examined by western blot analysis. The level of NF-κb phospho-p65 and IkBα protein expression were significantly up-regulated in ***LU1501*** treatment group(Fig. 3**D–F**). These results revealed that ***LU1501*** inhibited the cell growth and proliferation of breast cancer by suppressing the EGFR/NF-κb pathway.

2.2.3. *LU1501* inhibits breast cancer growth in vivo

To further study the anti-tumor effect of ***LU1501*** in vivo, tumor bearing mice model was established. SK-BR-3 tumor models were subsequently injected with saline, Erlotinib, Afatinib or ***LU1501*** (in the dosage of 1.2 mg/kg/day) within two weeks of continuous intraperitoneal injected (group = 4, n = 6). The mice were sacrificed after 14 days of administration of saline, Erlotinib, Afatinib or ***LU1501***. Tumor volume and weight were analyzed to determine tumor growth rate. Tumor volume of ***LU1501*** group was notably smaller than that of Erlotinib and Afatinib group (Fig. 4**A**), while the ratio of tumor and body weight of ***LU1501*** group was significantly less than that of Erlotinib and Afatinib groups (Fig. 4**B**).

Moreover, HE analysis, Tunel analysis and cell proliferation associated protein Ki-67 were analyzed by IHC staining (Fig. 4**C**). Graded scoring results were shown as Table 3. After administration in each group,. The necrotic areas of tumor cells in Elotinib, Afatinib and LU1501 groups were higher than those in PBS group. The infiltration degree of inflammatory cells in Elotinib group was significantly higher than that in other groups. Fibrosis in the necrotic area of the tumor can be seen in Afatinib group and LU1501 group.

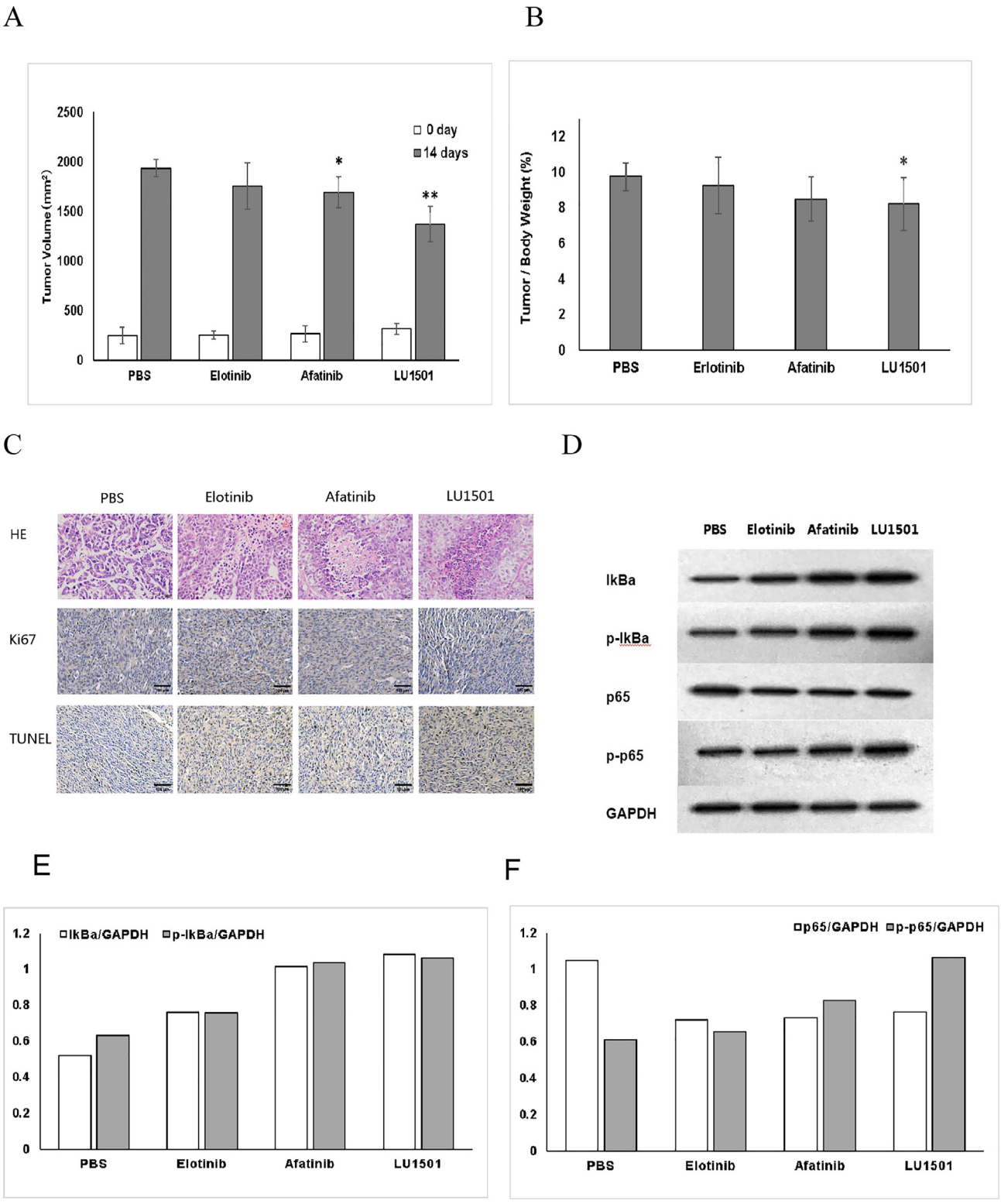


Fig. 4 (A) Tumor-bearing mice of different groups. (B) Tumor volumes after 14 days treatment. (C) HE, Tunel analysis and protein Ki-67 was analyzed by IHC staining. (D) The expression levels of EGFR/ NF-κb pathway related proteins in vivo were evaluated using Western blot analysis. *$p < 0.05$, **$p < 0.01$ when compared with control group. (E) Corresponding grayscale values analysis of EGFR/ NF-κb p65 and p-p65. (F) Corresponding grayscale values analysis of EGFR/NF-κb IkBα and p-IkBα.

Table 3 Graded scoring of HE.

Group	Score
PBS	1
Elotinib	3
Afatinib	2.5
LU1501	2.5

The finding was quite in agreement with in vitro results. The protein level of NF-κb phospho-p65, and IkBα similarly increased in ***LU1501*** group in vivo (Fig. 4**D–4F**). In summary, ***LU1501*** could inhibit the breast cancer cell proliferation and invasion both in vitro and in vivo.

2.3. Molecular docking study

In order to test the interaction and the binding modes of ***5a*** (***LU1501***) with the functional protein, the docking study of the compound with the p65 (PDB: 1NFI) and IkBα (PDB: 1IKN) protein were scored by Discover Studio 2020. The results showed that ***LU1501*** interacted with p65 protein mainly via hydrogen bond interaction (ARG30, ASP80, ARG164), hydrophobic interaction (PRO81, PRO82), strong π - π stacking hydrophobic interaction(PHE184) and halogen interaction between F atom and ASN190(Fig. 5). For the interaction between ***LU1501*** and IkBα protein, hydrogen bond interaction (GLN107, LYS323, PHE106, ASN105, CYS135, ALA133 and HIS171), hydrophobic interaction (PHE106, LEU104, CYS135 and PRO137) and halogen interaction between F atom and ASP136 were the main effects (Fig. 6). According to the binding pattern and chemical structure of ***LU1501***, we speculated this compound was a potential NF-κb inhibitor.

3. Discussion

The pathological morphology of breast cancer is complex. Usually, there are various types, and even more than two types may exist in the same cancer tissue or the same section (Calderon et al., 2020). In recent years, with the application of microarray technology and multi-gene RT-PCR quantitative detection method, breast cancer was divided into four types: triple negative breast cancer (ER-/PR-, HER2-), luminal A (ER + /PR + , HER2-), Luminal B (ER + /PR + , HER2 +) and HER2 + (ER-/PR-/HER2 +) (Weinberg et al., 2020; Akhtar et al., 2017; Chow et al., 2020; Stanley et al., 2015; Hamed et al., 2017). With the development of signaling pathway, apoptosis and other molecular biological methods in tumor research, molecular targets and targeted therapy of breast cancer have gradually become the trend and hotspot of anti-breast cancer research.

EGFR-Tyrosine Kinase Inhibitor (EGFR-TKI) blocks the EGFR signaling pathway by competitively binding to adenosine triphosphate (ATP) in the EGFR tyrosine kinase region (Ji et al., 2016). According to binding characteristics and action sites of different drugs, EGFR-TKI can be divided into three generations. The first generation of EGFR-TKI reversibly inhibits the tyrosine kinase activity of EGFR, and the representative drugs are Gefitinib and Erlotinib (Li et al., 2020; Liu et al., 2021). The second generation of EGFR-TKI such as Afatinib could irreversibly inhibit the tyrosine kinase activity of EGFR and other members of ERBB family as well (Takeda et al., 2021). The third generation of EGFR-TKI is characterized by effective mutation of EGFR 20 exon T790M, a common drug resistance target of the first and second generation of drugs, and it is easier to penetrate the blood–brain barrier (Tamiya et al., 2021). The representative drugs are oxitinib, amitinib and vometinib. However, the core structure of both amitinib and vometinib is similar to that of oxitinib, and the main purpose of the optimizing the structure is to further improve the binding of the drug to the mutant EGFR (Jiang et al., 2019; Roskoski, 2019; Wu et al., 2021). Alternatively, drugs can be developed with higher selectivity, that is, they can inhibit only mutant EGFR and have little inhibition on wild-type EGFR without mutation, thus reducing adverse reactions and improving safety (Wang et al., 2015; Pan et al., 2016).

From the structure and the protein binding prediction, ***LU1501*** is more selective in binding to EGFR receptors than erlotinib and afatinib. In vitro and in vivo breast cancer cytotoxicity tests also showed stronger inhibitory effects than these two positive drugs, especially in SK-BR-3 cells which were HER-2 and EGFR positive.

The intracellular signal transduction of EGFR is mainly through cytoplasmic MAPK, PI3K, c-SRC and nuclear NFκB signal pathway. In this study, it was found that ***LU1501*** can competitively bind to the ATP site of EGFR, blocking the transmission of its downstream signal pathway, especially inhibiting the activation of NF-κB/p65 and IκBα proteins. It is worth noting that molecular mRNA profiling results and signal pathway experiments showed that ***LU1501*** inhibited SK-BR-3 as well as MAPK signal pathway. Therefore, the regulation mechanism of ***LU1501*** on NF-κb, together with the pathway crosstalk between NF-κb and MAPK responding to ***LU1501*** may need further study in the future.

4. Conclusions

In this study, we designed, synthesized a series of novel 4-aminoquinazoline derivatives and also evaluated the biological activity against breast cancer by NF-κb pathway. From the MTT assay, most of the tested target compounds were found more effective towards SK-BR-3 than towards other three human breast cancer cell lines. ***LU1501,*** the most active compound among the tested derivatives exhibited comparable or lower IC_{50} values than control drugs on all the tested cells, suggesting ***LU1501*** had high inhibitory activity. In vivo studies were carried out to determine the activity of ***LU1501*** against breast cancers. After ***LU1501*** were injected into SK-BR-3 tumor bearing mice for 14 days, tumors were obviously inhibited. HE analysis, Tunel analysis and cell proliferation associated protein Ki-67 were analyzed by IHC staining, and the results further verified that ***LU1501*** appeared inhibitory effect through the NF-κb signal pathway by adjusting p65 and IkBα proteins. Later, the molecular docking studies were performed and the compound ***LU1501*** was found to bind well towards the proteins of p65 and IkBα on the NF-κb pathway, which were consistent with the biological data. Accordingly, ***LU1501*** was considered as a preferable and promising anti-tumor drug candidate, which could be used for further investigation in clinics.

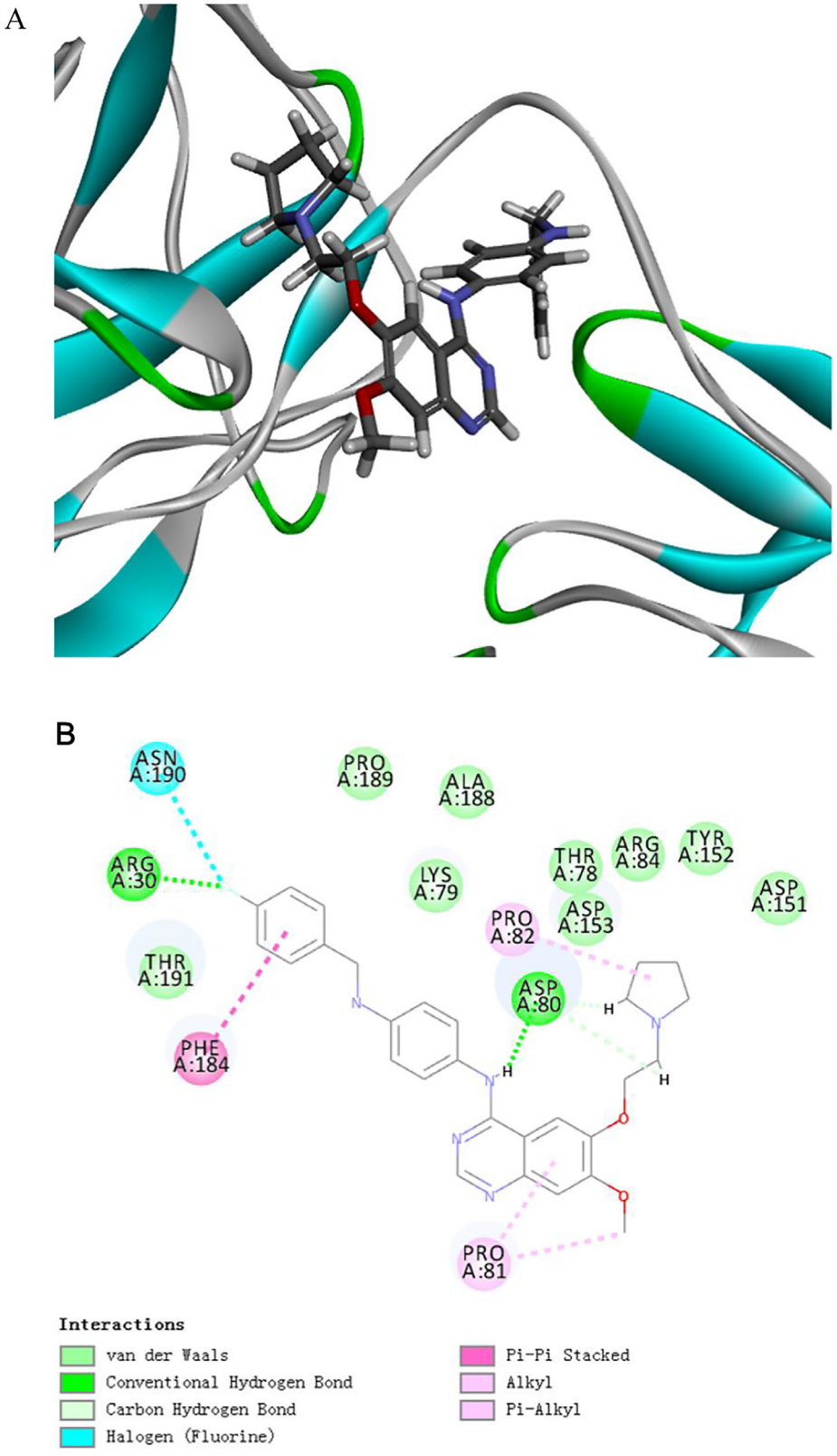


Fig. 5 Representations of lowest energy docking poses of ***LU1501*** bound to p65 proteins. (A) 2D interactions (B) 3D interactions.

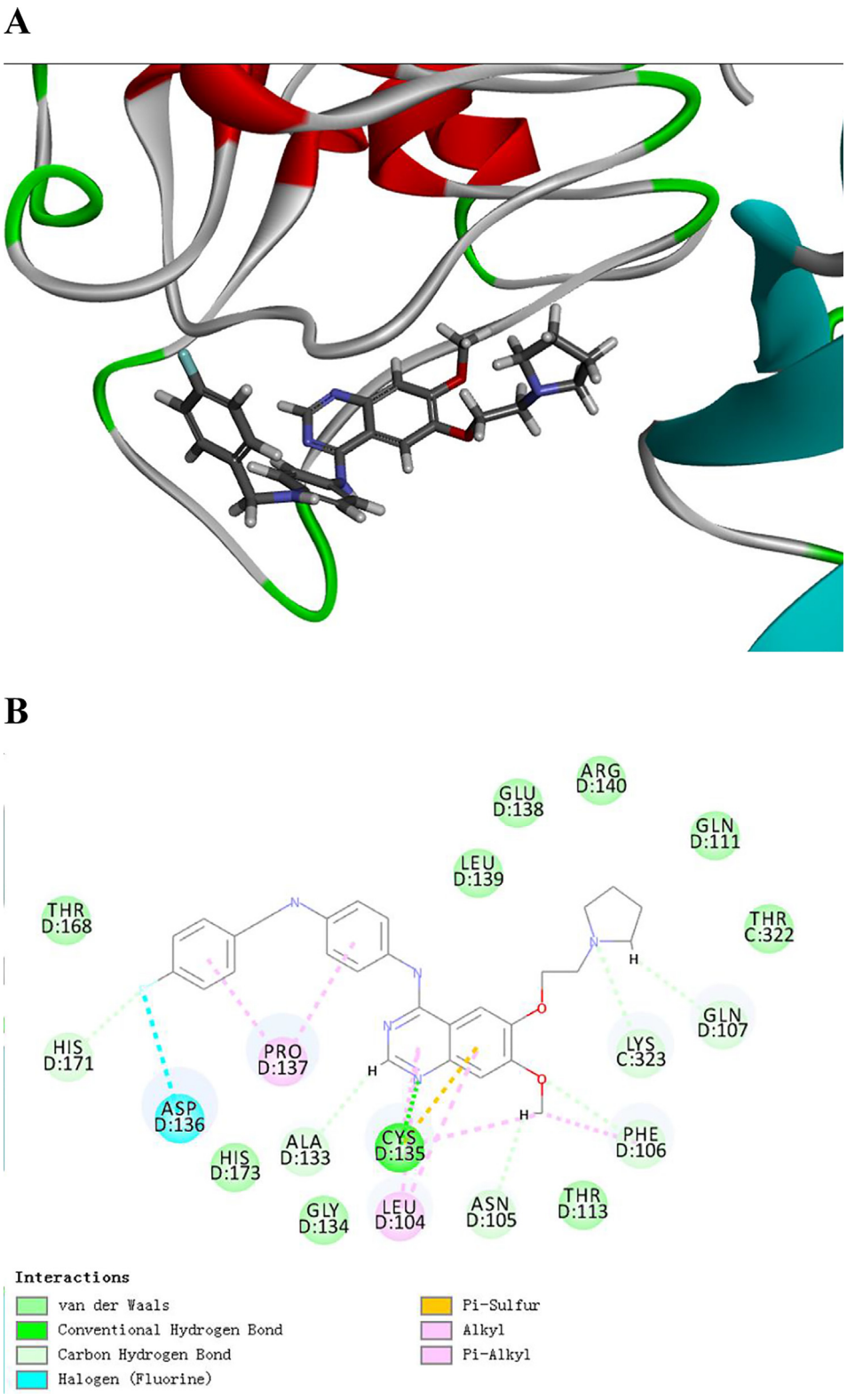


Fig. 6 Representations of lowest energy docking poses of ***LU1501*** bound to IkBα proteins. (A) 2D interactions (B) 3D interactions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.103908.

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